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Structure and developmental changes in the tergal glands of adult females of *Coptotermes formosanus* (Isoptera, Rhinotermitidae)

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Abstract

Female alates of the Formosan subterranean termite *Coptotermes formosanus*, possess a pair of glands under the 9th and 10th abdominal tergites. These tergal glands located just below the cuticle have two distinct regions. The outer part is made up of type 1 cells. These cells possess large nuclei, abundant mitochondria and bundles of microtubules. Apically the cells possess a distinct layer of microvilli. Numerous ducts with thick cuticular walls are seen traversing this region and the cuticle. The basal two thirds of the gland is composed of glandular cells which in the post-swarming female are packed with electron dense granules closely associated with mitochondria. The basement membrane has several conspicuous invaginations giving the gland a segmented appearance. In newly molted females, the glandular area lacks the dense granules but instead has electron lucent granules. Following swarming the alates lose their wings, a male and a female form a nuptial chamber, mate and lay eggs. In 7–10 day old females, the dense granules coalesce forming larger granules that appear to move towards the area of the intersegmental membrane for possible release. Also the cells appear to degenerate and large number of vacuoles appear throughout the gland.
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Keywords: Tergal glands; Termite; Glandular cells; Secretion

1. Introduction

In most termite species, swarming, which is essentially a dispersal flight, is followed by sexual behaviors that culminate in a pair in tandem finding a suitable nest site to establish a new colony. Secretions from three types of glands, the sternal glands, posterior sternal glands and tergal glands are known to be involved in the post-flight sexual behaviors (Pasteels and Bordereau, 1998; Quennedey et al., 2004). Ampion and Quennedey (1981) investigated the distribution of these glands in 100 termite species. The sternal glands produce a trail pheromone which in a few species may also act as a sex pheromone when present in higher quantities and during the act of calling (Pasteels and Bordereau, 1998). The posterior sternal glands have been reported in both male and female alates of a number of termite species (Ampion and Quennedey, 1981). The tergal glands may be present in alates of either or both sexes and their number also vary with species. According to Ampion and Quennedey (1981), seven genera in the family Rhinotermitidae possess tergal glands ranging in number from 2 to 3. Their secretions and precise role in sexual behavior remain largely undetermined.

Tergal glands were first reported by Montalenti (1928) in Kalotermitidae. Barth (1955) described a pair of tergal glands on 9th and 10th tergites of females of *Syntermes dirus*. He further suggested that the glands, with two types of cells, produced lipid or lipid-soluble substances that appeared to have an effect on the pairing of the reproductives after the flight. Noirot (1969) discussed the distribution and possible function of these glands in several families of termites. In some species of termites such as *Trinervitermes bettonianus* (Leuthold, 1977), and *Cornitermes bequaerti* (Bordereau et al., 2002), the tergal glands in the female are reported to produce a pheromone which evokes long-range attraction.

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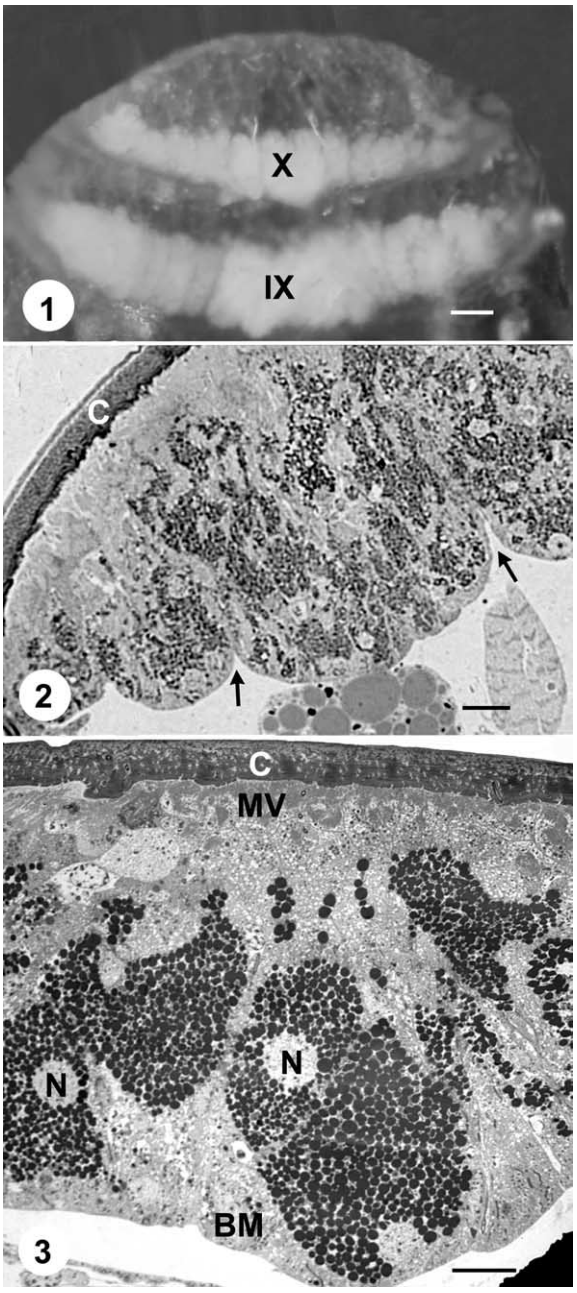
The Formosan subterranean termite, *Coptotermes formosanus*, is a major urban pest in several southern states and Hawaii in the United States as well as in Far East (Raina et al., 2001). In Louisiana, the adults swarm between April and June. Following a brief flight to the nearest light source, the alates land on the ground and shed their wings. The females of this species do not call and the first contact between the sexes is incidental after which the male follows the female in tandem until they find a suitable nesting site (Raina et al., 2003a). During the tandem behavior, the male using its cephalic appendages, maintains close contact with the tip of the female abdomen. The females of *C. formosanus*, possess a pair of tergal glands under the 9th and 10th abdominal tergites. The major component in the extracts of tergal glands was recently identified as the triacylglycerol trilinolein (Bland et al., 2004).

We report on the structure of the tergal glands in *C. formosanus*, including developmental changes and speculate on possible mechanisms for the release of their secretion.



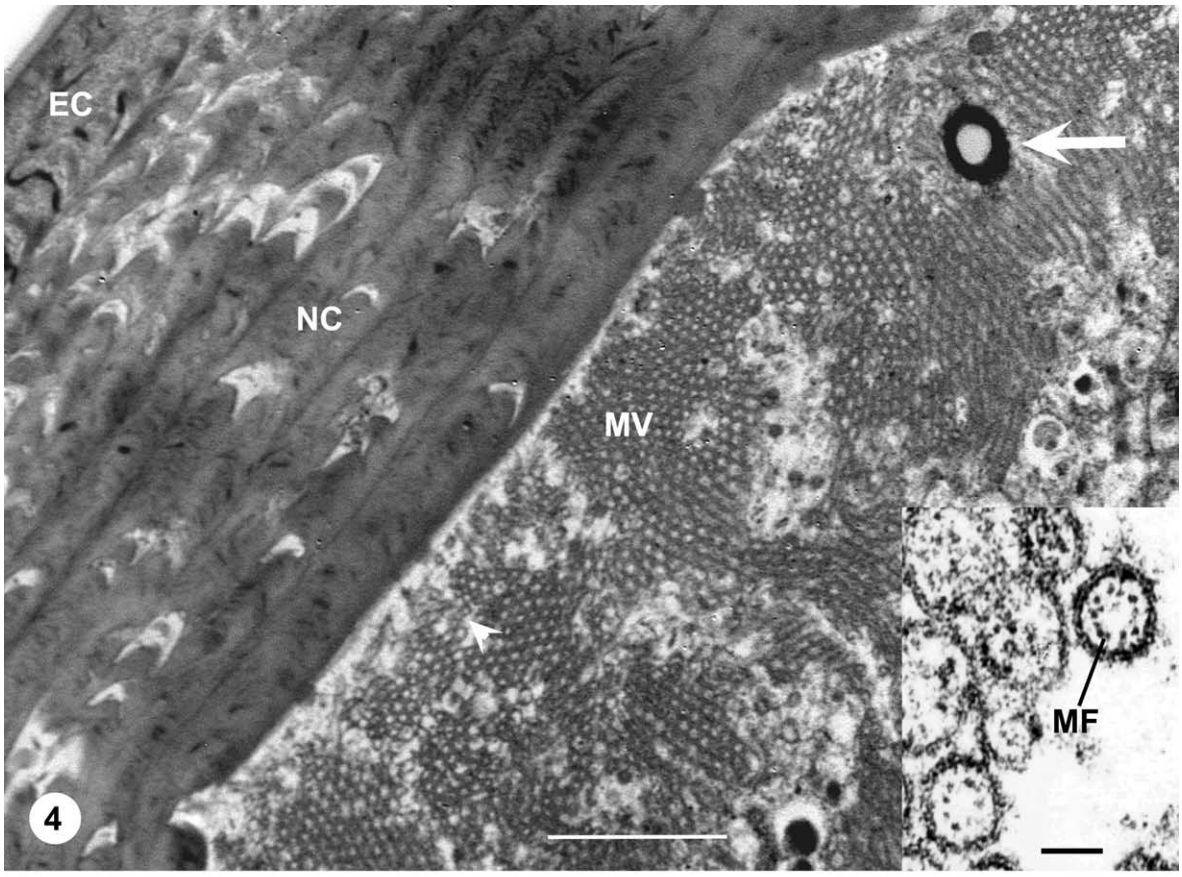
Figs. 1–3. Fig. 1: a pair of tergal glands attached to the underside of the 9th and 10th abdominal tergites in female alates of *C. formosanus* (bar 100 μm). Fig. 2: longitudinal section of a part of the epoxy embedded tergal gland from the 9th segment of a post-flight female. The gland is dorsally covered by the cuticle and ventrally the basement membrane has invaginations (arrows) (bar 10 μm). Fig. 3: an electronmicrograph of a cross section through the tergal gland of a post-flight female showing the cuticle. Just below the cuticle is a layer of microvilli (MV). Cells in the glandular area have round nuclei and are packed with dense granules. A basement membrane encloses the gland on the ventral side (bar 10 μm). Abbreviations used: BM, basement membrane; C, cuticle; MV, microvilli; N, nucleus.

2. Materials and methods

Alates of *C. formosanus* were collected in light traps following swarming between April and June in New Orleans, Louisiana. Pairs were set up in Petri dishes as reported earlier (Raina et al., 2003b). Newly molted pre-flight alates were obtained either from termite infested landscape ties or from final instar nymphs collected from field and maintained in the laboratory.

For light microscopy, abdomens of pre-flight, immediately after flight and 7–10 days old mated females were excised around the middle and fixed in Carnoy's fixative for 2 h. The paraffin blocks were serial sectioned at 5 μm thickness, sections were stained with Mallory's triple stain and examined with an Olympus BX60 microscope. For electron microscopy, 8th through 10th abdominal segments of the females were cut and the 9th and 10th tergites excised with fine scissors. After removing extraneous fat and other tissues, small sections of the tergites together with the attached tergal glands were cut and immediately fixed in Karnovsky fixative for 1 day. Tissues were then washed in 3–4 changes of 0.05 M phosphate buffer, post-fixed in buffered 2% osmium tetroxide for 2 h, dehydrated in a graded ethanol series, and embedded in Spurr's low viscosity medium. Sections (1 μm thick) were stained with methylene blue and examined under the light microscope. Ultrathin sections were cut on a Reichert/AO Ultracut microtome with a diamond knife. The sections were stained with 2.5% uranyl acetate for 1 h, followed by 3% lead citrate for 5 min. Sections were viewed with a Philips CM-120 transmission electron microscope operated at 80 kV.

Surface of the tip of female abdomen was examined using low temperature scanning electron microscopy (Wergin et al., 2000), in a Hitachi S-4100 field emission SEM.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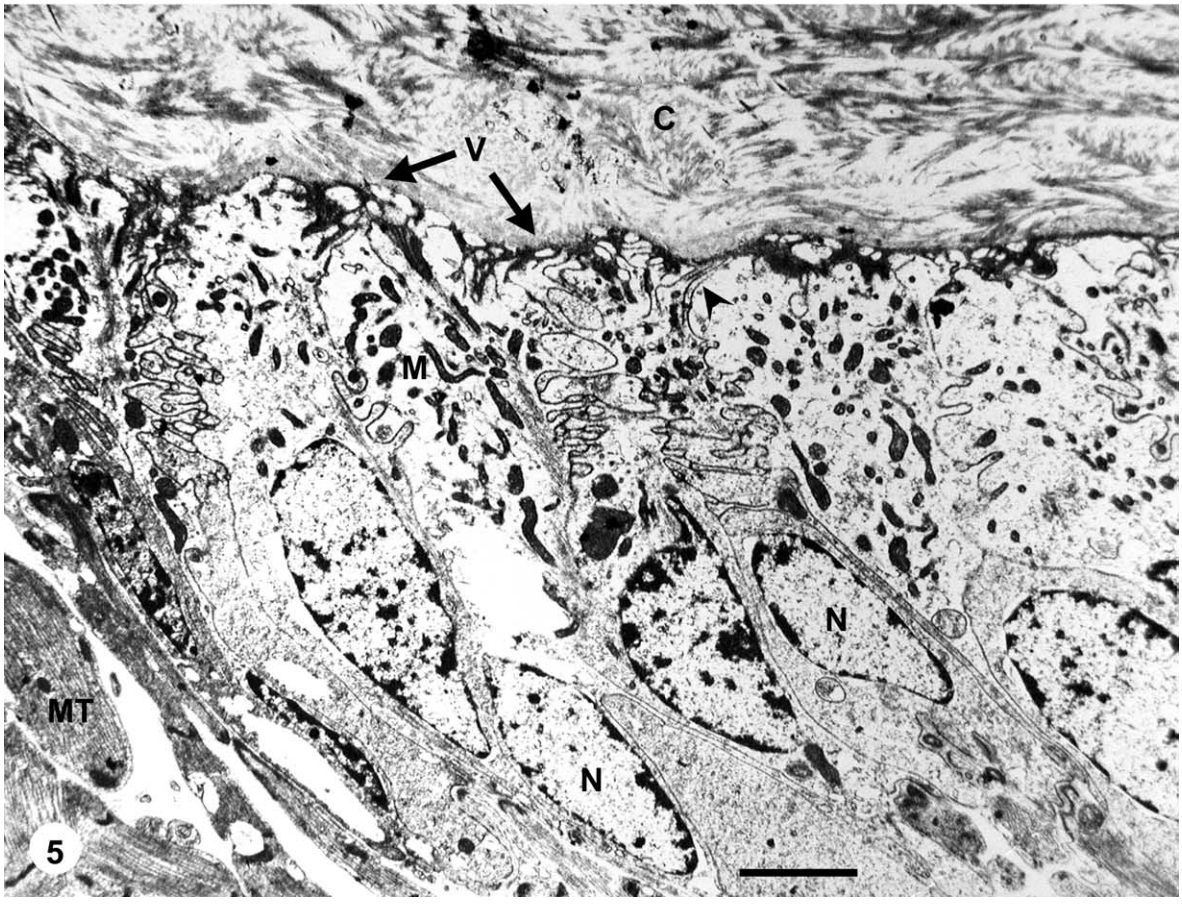
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3. Results

A pair of tergal glands are located under the 9th and 10th tergites of adult females of *C. formosanus*. The gland of the 9th tergite is larger, approximately 1.1 mm long and 0.18 mm at its widest, whereas the one under the 10th tergite is only 0.76 long (Fig. 1). Deep invaginations in the basal region give the gland a segmented appearance (Fig. 2). The glands of the 9th and 10th tergites have 17 and 11 segments respectively, and each gland has two distinct regions, with the glandular region occupying the basal area.

At the ultrastructural level, the glands appear to be directly attached to the cuticle, which is about 6 μm thick (Fig. 3). The cuticle has a fine lamellar structure and is well differentiated into a thin epicuticle on the outside followed by the exocuticle and endocuticle (Fig. 4). The pre- and post-ecdysial endocuticle is made up of 9 and 5 lamellae respectively. Pore canals running through the cuticle are sparse but vertical striations are common (Fig. 4). The cellular layer immediately below the glandular cuticle is composed of type 1 cells. These type 1 cells are elongated with large centrally placed oblong nuclei having the chromatin dispersed along the nuclear membrane (Fig. 5) and a distinct apical layer of microvilli (Figs. 3 and 4) with longitudinally arranged microfilaments (Fig. 4 inset). Ducts (0.5 μm dia) with thick walls that appear to be derived from epicuticle are confined to the apical region of the gland. The cells also have numerous mitochondria and bundles of microtubules associated with them. Many vesicles are located just below the cuticle.

The glandular area, which makes up about two thirds of the gland, consists of cells with round basal nuclei (Fig. 6). The cells are between 35–40 μm at their widest and their nuclei are 8–9 μm in diameter. There is no chromatin accumulated along the nuclear membrane. In the post-flight females, these cells are full of electron dense granules measuring an average of 0.9 μm in diameter (Fig. 6). The cytoplasm of the glandular cells is very rich in mitochondria and rough endoplasmic reticulum. The mitochondria are often seen enclosing the dense granules (Fig. 7). Another type of smaller cells is found near the base of the gland. These cells are elongated, the cytoplasm has no dense granules but contains large number of small vesicles.

In the pre-flight females, the cells in the glandular portion lack electron dense granules. Instead they have electron lucent granules that are also fewer in number (Fig. 8). These granules are also smaller (0.6 μm dia) than the electron dense type. Associated with the lucent granules are found large numbers of vesicles (0.3 μm dia) and mitochondria. Chromatin in the nuclei of these cells is dispersed along the nuclear membrane. In 7–10 day old mated females, the dense granules appear to coalesce (Fig. 9). In addition, very large number of vacuoles appear in the cells of the apical region, mitochondria and nuclei disappear and groups of microvilli show signs of breaking up (Fig. 10). The dense granules move closer to the cuticle and through coalescence form very large granules measuring 5–6 μm in diameter (Fig. 11). Towards the anterior and posterior edges of the gland (closer to the intersegmental membranes) the cells show a complete break down possibly affecting the release of the granules.

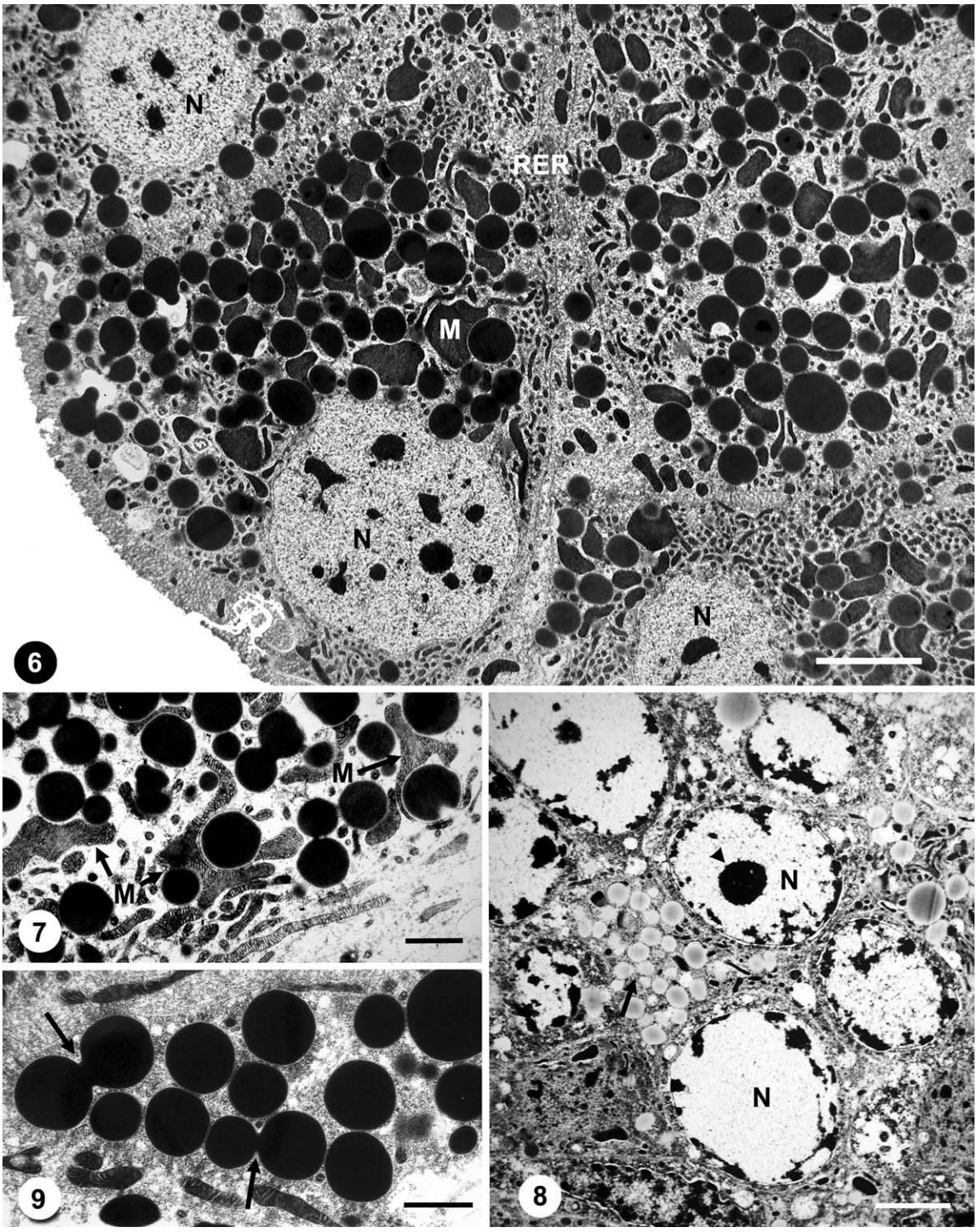
The surface of the cuticle in the 9th and 10th tergites above the tergal glands shows fine pores about 0.4 μm in diameter and 35–40 μm apart located in depressions. These pores are present along the anterior margin of each of these tergites (Fig. 12). Normally this area is covered by the intersegmental membrane and the posterior edge of the tergite from the preceding segment.

4. Discussion

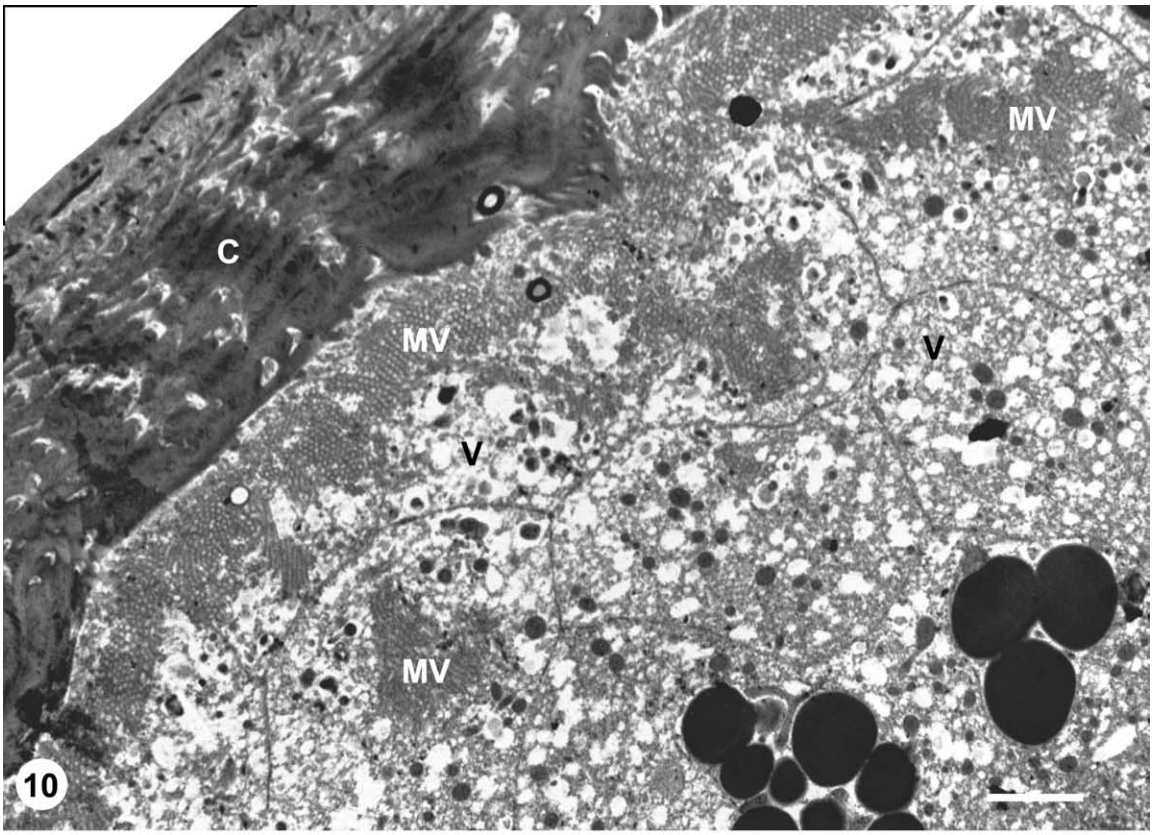
In *C. formosanus*, a pair of tergal glands present under the 9th and 10th tergites of females has been associated with post-flight sexual behavior (Raina et al., 2003a; Park et al., 2004). We observed a marked difference between the structure of the tergal glands from pre-flight and post-flight females and finally of females that had been paired with males for 7–10 days. The conspicuous electron dense granules seen in the post-flight female glands were absent in glands from pre-flight females. Whereas, in the older females, the granules coalesced to form large dense granules accompanied with cell degeneration.

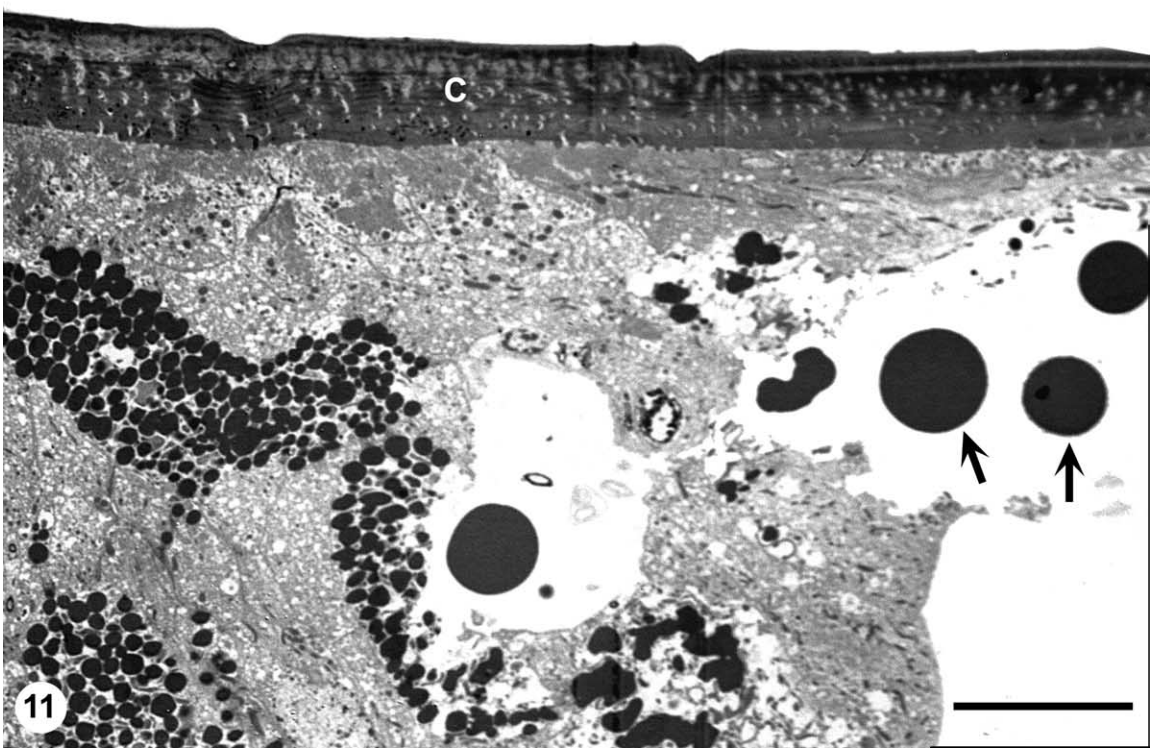
Based on structure, the cells in the epidermal glands in insects have been categorized into 3 classes (Noirot and Quennedey, 1974, 1991; Quennedey, 1998). The apical region of the tergal glands in *C. formosanus* is made up of class 1 cells with active, centrally located nuclei. These cells are devoid of any dense granules. However, Bordereau et al. (2002) reported the presence of many dense granules in the apical class 1 cells in tergal glands of *C. bequaerti*. The class 1 cells are characterized by the presence of an apical border of microvilli (Quennedey, 1998), thereby increasing the cell surface. The microvilli in tergal glands of *C. formosanus* contain longitudinal microfilaments arranged in a ring around an axial core. These cells also have ducts lined with epicuticle whose diameter corresponds to that of the pores seen in the cuticle above the gland. It is speculated that these

Figs. 4 and 5. Fig. 4: structure (TEM) of the apical region of a class 1 tergal gland cell. Cuticle is distinguishable into a thin epicuticle, exocuticle and endocuticle. Below the cuticle is a layer of microvilli and a duct (arrow) in a cross-section (bar 2 μm). Arrow head (white) marks the area shown in the inset. The inset shows microvilli in cross section with a ring of microfilaments (bar 100 nm). Fig. 5: tangential section through class 1 cells. Dorsal part of the cytoplasm of these cells has convoluted margins leading into narrow tubes ending below the cuticle (arrow head, black) surrounded by many vesicles. The cells as well as the nuclei are elongated and there are many mitochondria. There are also bundles of microtubules (bar 2 μm). Abbreviations used: C, cuticle; EC, exocuticle; M, mitochondria; MF, microfilaments; MT, microtubules; MV, microvilli; N, nucleus; NC, endocuticle; V, vesicles.



Figs. 6–9. Fig. 6: basal glandular area showing cells packed with electron dense granules, rough endoplasmic reticulum and mitochondria (bar 2 μm). Fig. 7: the large mitochondria appear to encircle the dense granules (arrows) (bar 1 μm). Fig. 8: section through the glandular area of newly molted female alate. The nucleoli (arrow head) are large and the chromatin is dispersed along the nuclear membrane. Cytoplasm contains electron lucent granules (arrow) (bar 2 μm). Fig. 9: in glandular cells of 7–10 day old mated females, the dense granules appear to coalesce (arrows). Abbreviations used: M, mitochondria; N, nucleus; RER, rough endoplasmic reticulum.





Figs. 10 and 11. Fig. 10: sections through apical portion of the tergal gland of a 7–10 day old mated female. The cells show very high vacuolation, lack of nuclei and breakdown of microvilli (bar 2 μm). Fig. 11: sections through apical portion of the tergal gland of a 7–10 day old mated female. Some of the dense granules have moved closer to the cuticle, cell disruption is more advanced and the coalesced granules (arrows) appear to be released towards the intersegmental membrane (bar 10 μm). Abbreviations used: C, cuticle; MV, microvilli; V, vacuoles.

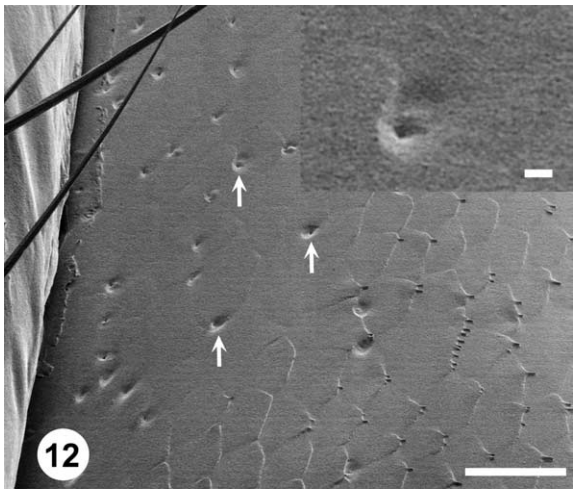


Fig. 12. SEM of the anterior region of 9th tergite above the tergal gland showing tiny pores (arrows) located in pits or depressions (bar 30 μm). One of the pores is magnified in the inset (bar 2 μm).

ducts may be carrying secretions from class 1 cells to the exterior. The nature of the secretion produced by these cells in *C. formosanus* is unknown.

The basal two thirds of the gland in *C. formosanus* is composed of glandular cells without any apparent structural specialization for the release of their products. Ampion and Quennedey (1981) reported that the tergal glands in the genus *Coptotermes* consist of class 1 and class 3 cells. Barth (1955) described two types of cells in the tergal glands of the termite *S. dirus*, with the larger glandular cells having two nuclei and a system of ramifying canaliculi, to communicate with the exterior. Bordereau et al. (2002) referred to the secretory cells in the tergal glands of *C. bequaerti* as being of class two type. Structural organization of the glandular cells in the basal region of tergal glands in *C. formosanus* does not support their categorization as type 2 or 3 cells.

The main structural feature of the tergal glands in post-flight *C. formosanus* females is the presence of very large number of electron dense granules. These granules are absent in the newly molted (pre-flight) females. Glandular cells in the pre-flight females have very active nuclei and the cytoplasm contains lucent granules. Considering that the extract of tergal glands from post-flight females contains a relatively large amount of trilinolein (Bland et al., 2004), it is safe to assume that the dense granules may be the source of this compound. Lucent granules could then represent as containing a precursor of trilinolein. The deep invaginations in the basal lamina are possibly the avenues for the uptake of a putative precursor from hemolymph. The lucent granules would then undergo a process of maturation in association with the mitochondria. In fact the granules are often found enclosed by large mitochondria. Noirot and Quennedey (1974) while describing various cell types in the epidermal glands also reported that in glandular cells, mitochondria were found in close contact with clear vesicles as well as dense granules. Roth (1969) in a study of cockroach tergal glands reported that immature lipid droplets give rise to electron dense lipid granules. However, in the epidermal glands of neotenic reproductives of *Prorhinotermes simplex*, Šobotník et al. (2003) reported that dense granules are transformed into lucent type before being released.

In older mated females the dense granules coalesce to form larger granules and with the disruption of cells these appear to be released towards the intersegmental membranes. In the absence of canals and processes commonly associated with the release of secretory products of class 3 glandular cells, it is presumed that the lipoidal material from the tergal glands may be transported across this membrane. It was recently reported that in *C. formosanus*, the tergal glands degenerate as the females age (Park et al., 2004).

While as the association of sternal glands of termites has been clearly linked to trail and calling/sex pheromones in worker and adult castes respectively, the function of tergal glands is ambiguous. In at least two cases the tergal glands have been implicated in the production of sex pheromones and calling. Bordereau et al. (2002) reported that the tergal glands in *C. bequaerti*, produce (3*Z*, 6*Z*, 8*E*)-dodecatrien-1-ol, known as both a trail and sex pheromone in several species of termites. Quennedey et al. (2004) while describing the ultrastructure of posterior sternal glands in *Macrotermes annandalei*, also referred to the tergal glands as being involved in calling behavior. In this species, both types of glands have pouches that open to the outside through pores in the cuticle. These pouches, filled with secretory products, collapse after the termination of calling. In *C. formosanus*, the female alates, after they swarm and drop their wings, do not call. Because of the large numbers of individuals present in a swarm, first contact between sexes is accidental with no apparent involvement of a long range sex pheromone (Raina et al., 2003b). It was further suggested that the tergal glands may be producing a contact sex pheromone that mediates tandem behavior with the male maintaining contact with the tip of female abdomen. During the tandem behavior, the female abdomen is slightly swollen resulting in fully exposing the intersegmental membranes above the tergal glands (Park et al., 2004). The males probably acquire orally the material during tandem running and subsequent cohabitation with the female. The males of this species do not have any trilinolein at the time of swarming, but slowly acquire it perhaps as a companionship gift (Park et al., 2004). In cockroaches, generally the males possess the tergal glands. In *Blattella germanica*, females were reported to feed on the tergal gland secretions from the male tergites (Roth, 1969). Recently, the phagostimulant component in the male secretions in this case was shown to be composed of complex mixture of oligosacchrides and phospholipids (Nojima et al., 2002). However, the exact function of tergal glands and their secretion in *C. formosanus* remains to be determined.

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